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Declaration

I, Makoto Asashima, who belongs to University of Tokyo, Graduate School of Arts and Sciences, Multi-Disciplinary Sciences, Life Sciences, at 3-8-1, Komaba, Meguro-ku, Tokyo Japan, say solemnly and faithfully in a deposition as follows:

1. I was born on September 6, 1944, at Sado, Niigata; after graduating from University of Tokyo, School of Science, Graduate Department, in 1972, I belonged to Institute of Molecularbiology, Free University of Berlin, Germany, in 1972; then worked for Yokohama City University, Faculty of Arts and Sciences as an assistant professor from 1974, and as a professor from 1985; worked for University of Tokyo, College of Arts and Science, as a professor from 1993; for University of Tokyo, Graduate School of Arts and Sciences, Multi-Disciplinary Sciences, Life Sciences, as a professor and a council from 1996 to present.

2. I have been involved mainly in study of developmental biology, organogenesis, molecular biology, and differentiation and regeneration. My brief history is attached to this declaration together with the list of references including my works in the last decade, as separate sheet I.

3. As shown in my brief history, I am a council of Japanese Society of Development Biology and of Japanese Society of Cell Biology, and a member of Japanese Society of Molecular Biology. Further, I have been commended for achievements in the field of developmental biology (see attached "A Brief History").

4. I am an editor of "Develop. Growth Differ", "Int. J. Dev. Biol", and "Zool. Sci." These magazines are comprised of study reports in the field of developmental biology.

5. I was asked to consider the following points:

(i) The Examiner admits as follows: "The claims are drawn to using genomic markers to determine the stage of organ development, the gene expression in various stages of the development in different organs are bound to be distinct among different classes of vertebrate, the specification fails to teach which of genes expressed at what stage in which organ of the which vertebrate could be used as a determining factor for amphibians, birds, bony fish, chimaera chondrichthian, mammal, reptile, etc., one skilled in the art could not practice the claimed invention without first carrying out

extensive experimentation to determine the parameters for practice the invention, i.e. which genome DNA could be used for stage markers for a particular organ in a particular vertebrate animal."

However, for practice of the invention, in case a particular organ in a particular vertebrate is targeted, the person skilled in the art can easily (without undue experimentation) determine which gene DNA can be used as stage markers, by ordinary methods such as the differential display method. For researchers in this field, the opinion 'As to all of amphibians, birds, bony fish, chimaera chondrichthian, mammal, reptile, etc., one skilled in the art could not practice the claimed invention without first carrying out all of the extensive experimentation.' is unacceptable."

(ii) The Examiner also admits as follows: "In view of the quantity of experimentation necessary to determine the parameters for achieving *in vitro* induced, stage-specific organ preparation, in particular for obtaining organs functioning *in vivo* when transplanted into a recipient of the same species, the lack of guidance provided by the specification as well as the absence of working examples with regard to *any* organ from *any* species of a vertebrate, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention."

However, as in the description of "the basic rule of body formation is common to all the vertebrates and homologous genes are known to have quite a similar function among different species." in the specification, the principle of basic differentiation such as development, cell differentiation, organ differentiation, and so on, is common to all vertebrates, and the same is true of different species.

(iii) The Examiner admits that Asashima et al. anticipates the instant claims, indicating that "Asashima et al. teach *in vitro* induced organs, such as notochord, muscle, mesenchyme, and epidermis (Fig. 2). The organs are induced from early Xenopuse (vertebrate) animal-cap cells (ectoderm of a stage 9 blastula), wherein the cell explants are cultured in the presence of activin. Asashima et al. teach that different genes may express at different stages of the embryo development, such as Xar9 (EDF, left column of page 6512)".

The Examiner also admits that Ariizumi et al. anticipates the instant claims, indicating that "Ariizumi et al. teach *in vitro* induced organs, such as notochord, muscle, mesenchyme, blood cells, and epidermis. The organs are induced from presumed ectoderm region of Xenopuse (see Materials and Methods on page 412), wherein the cell explants are cultured in the presence of activin."

Further, the Examiner admits that Asashima et al. anticipates the instant claims, indicating that "Asashima et al. teach that treatment of amphibian explants with

activin A led to differentiation of mesodermal derivatives such as notochord, muscle, mesenchyme, and blood cells (abstract, figs. 1,3, table 1)".

However, when I wrote these papers, I did not think about transplantation at all. Further, at that time, it would have never occurred to researchers in this field who read this paper that transplantation could be conducted when the developmental stages of recipients and explants are corresponding.

(iv) The Examiner admits that the claimed invention as a whole was obvious, stating "It would have been obvious to one of ordinary skill in the art at the time the invention was made to apply the methods taught by Hullet et al. and Andersen et al. in evaluating the organs taught by Asashima et al. with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to use the method for evaluation of any cultured organ intended for use in transplantation because it is the reliable way to find out whether the cultured organ could function properly in vivo".

However, I do not understand the ground for the Examiner's admission. In particular, though he admits "The ordinary skilled artisan would have been motivated to use the method for evaluation of any cultured organ intended for use in transplantation because it is the reliable way to find out whether the cultured organ could function properly in vivo", such admission is far from the admission of researchers. I, as an researcher, do not understand the admission stating that the present invention is obvious based on the references which do not describe the cultivation wherein an organ induced from ectoderm region which had been cut out from blastula is cultured to the corresponding stage of the recipient vertebrate, even though transplantation is considered.



Makoto Ashashima

Date this 6 day of Sept, 2002

A Brief History

Name : Makoto Asashima, Ph.D.

Position : Professor

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Graduate School of Arts and Sciences
The University of Tokyo

Nationality: Japan

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Home address: 3-40-9 Minami-Otsuka, Toshima-ku, Tokyo 170-0005, Japan

Tel: +81-3-3981-0348

Birth : Born at Niigata (Japan) in 1944, Sept. 6

Education :

Graduated from Tokyo University for Education in March, 1967

Finished the graduate courses at the University of Tokyo

Master of Science from the University of Tokyo in March 1969

Doctor of Science from the University of Tokyo in March 1972

Professional career :

Research Associate of Institute of Molecularbiology, Free University of Berlin (Germany)
(April 1972-September 1974)

Associate Professor of Yokohama City University, Faculty of Arts and Sciences
(October 1974-December 1985)

Professor at Yokohama City University, Faculty of Arts and Sciences
(January 1985-March 1993)

Professor at The University of Tokyo, Faculty of Arts and Sciences
(April 1993-March 1995)

Professor at The University of Tokyo, Graduate School of Arts and Sciences
(April 1995-present)

Academic activity : (present, 2002)

Japanese Society of Developmental Biology (Vice-President)

Japanese Society of Zoological Science (Vice-President)

Japanese Society of Cell Biology (Council)

Japanese Society of Space Biology (President)

Japanese Society of Molecular Biology (Council)
International Society of Developmental Biologists (Academic council member)
New York Science Academy (Active member)
Tokyo University Press (Trustee)
Development Growth and Differentiation (Editor in Chief)
Cell Structure and Function (Editor)
Int. J. Developmental Biology (Associate editor)
Zoological Science (Editor)

Prizes

1. Prize of Japanese Society of Zoological Science in 1990
2. Prize of Inoue Academic Foundation (with gold medal) in 1990
3. Prize of Kihara Memorial Academic Foundation (Silver Flower design with gold frame) in 1994
4. Siebold Prize (Germany; President Weiszeker) in 1994
5. Toray Science Prize (with gold medal) in 1999
6. Mochida Medical Science Prize (with gold medal) in 1999
7. Naito Memorial Science Prize (with gold medal) in 2000
8. Prize of Japanese Society of Bioindustry in 2000
9. Uehara Prize (with gold medal) in 2000
10. Japan Academy Prize and Imperial Special Prize (with silver bottle) from Society of Japan Academy in 2001
11. Purple Ribbon Prize (Academic field called as Shiji-hosho) from the Japanese Government in 2001.
12. The prince Hitachi Prize for comparative oncology in 2002

Professional : Developmental Biology, Cell Biology

1. Mechanism of egg development using from experimental biology to molecular biology
2. Control of Organogenesis *in vitro*
3. Molecular biology of cell differentiation and embryonic development.
4. Cloning and analysis of organ specific genes and early embryonic development

Books :

1. Development and its Mechanism (Idemitsu Press, in 1983)
2. Modern biology (Riko-Gakusha, in 1988)
3. Developmental biology (Asakura-Press, in 1996)
4. Mechanism of Developmental Biology (Iwanami-Press, in 1998)
5. Fundamental of Molecular biology (Shokabo-Press, in 2000) Other 15 books

Papers : over 170 original papers

Hobby : book reading and walk

List of papers
(1990-2002)

Makoto Asashima
Professor
Department of Life Science (Biology)
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3-8-1 Komaba, Meguro-ku
Tokyo 153-8902, Japan

1. Mesodermal induction in early amphibian embryos by activin A (erythroid differentiation factor).
Asashima, M., Nakano, H., Shimada, K., Kinoshita, K., Ishii, K., Shibai, H., and N.Ueno
Roux's Archives Develop. Biol., 198, 330-335 (1990)
2. The vegetalizing factor belongs to a family of mesoderm inducing proteins closely related to erythroid differentiation factor.
Asashima, M., Nakano, H., Uchiyama, H., Davids, M., Plessow, S., Loppnow-Blinde, B., Hoppe, P., Dau, H., and Tiedemann, H.
Naturwissenschaften, 77, 389-391 (1990)
3. Observations on cardiac ultrastructure of the adult Japanese newt, *Cynops pyrrhogaster*.
Pfeiffer, C. J., Asashima, M., Cho, C. H., and Shimada, K.
J. Submicrosc. Cytol. Pathol., 22, 17-26 (1990)
4. Gastric leiomyomas in the Japanese newt, *Cynops pyrrhogaster*. Ultrastructural observation.
Pfeiffer, C. J., and Asashima, M.
J. Comp. Pathol., 102, 79-87 (1990)
5. Blood cell morphology and counts in the Japanese newt (*Cynops pyrrhogaster*).
Pfeiffer, C. J., Pyle, H., and Asashima, M.
J. Zoo and Wildlife Medicine, 21(1), 56-64 (1990)
6. Embryonic induction and cell differentiation during the amphibian development.
Asashima, M.
Zoological Science, 7, 1015-1016 (1990)
7. Concentration-dependent inducing activity of activin A.
Ariizumi, T., Moroiya, N., Uchiyama, H., and Asashima, M.
Roux's Archives Develop. Biol., 200, 230-233 (1991)
8. The vegetalizing factor from chicken embryos : its EDF (activin A)-like activity.
Asashima, M., Uchiyama, H., Nakano, H., Eto, Y., Ejima, D., Sugino, H., Davids, M., Plessow, S., Born, J., Hoppe, P., Tiedemann, H., and H.Tiedemann, H.

- Mechanisms of Development, 34, 135-141 (1991)

9. Presence of activin (Erythroid Differentiation Factor) in unfertilized eggs and blastulae of *Xenopus laevis*.
Asashima, M., Nakano, H., Uchiyama, H., Sugino, H., Nakamura, T., Eto, Y., Ejima, D., Nishimatsu, S., Ueno, N., and Kinoshita, K.
Proc. Nat. Acad. Sci., U.S.A., 88(15), 6511-6514 (1991)
10. Follistatin inhibits the mesoderm-inducing activity of activin A and the vegetalizing factor from chicken embryo.
Asashima, M., Nakano, H., Uchiyama, H., Sugino, H., Nakamura, T., Eto, Y., Ejima, D., Davids, M., Plessow, S., Cichoka, I., and Kinoshita, K.
Roux's Archives Develop. Biol., 200(1), 4-7 (1991)
11. Magnetic shielding induces early developmental Abnormalities in the newt, *Cynops pyrrhogaster*.
Asashima, M., Shimada, K., and Pfeiffer, J. C.
Bioelectromagnetics , 12, 215-224 (1991)
12. Dose and time-development mesoderm induction and outgrowth formation by activin A in *Xenopus laevis*.
Ariizumi, T., Sawamura, K., Uchiyama, H., and Asashima, M.
Int. J. Develop. Biol., 35(4), 407-414 (1991)
13. *Xenopus* cell line have inducing activities on presumptive ectoderm of *Cynops* embryo.
Moriya, N., Kudo, Y., Uchiyama, H., and Asashima, M.
J. Yokohama City Univ. (Natural Science), 5, 1-13 (1991)
14. Mesoderm induction in early amphibian embryos by activin A and its related gene products in *Xenopus laevis*.
Ueno, N., Asashima, M., Nishimatsu, S., Suzuki, A., and Murakami, K.
Elsevier Science Publishers B.V. Frontiers in muscle research, 17-28 (1991)
15. Nephroblastoma in the clawed frog *Xenopus laevis*.
Meyer-Rochow, V. B., Asashima, M., and Moro, S. D.
J. Exp. Anim. Sci., 34, 225-228 (1991)
16. Liver ultrastructure and a new cell type in the Japanese newt, *Cynops pyrrhogaster*.
Osman, A. H. K., Pfeiffer, C. J., and Asashima, M.
European Journal of Morphology, 29(4), 255-270 (1991)
17. Isolation and characterization of native activin B.
Nakamura, T., Asashima, M., Eto, Y., Takio, K., Uchiyama, H., Moriya, N., Arizumi, T., Yashiro, T., Sugino, K., Titani, K., and Sugino, H.
J. Biol. Chemistry, 267(23), 16385-16389 (1992)
18. Comparative study of sexual dimorphism of the innominate bone in rodents and amphibians.
Uesugi, Y., Ohta, Y., Asashima, M., and Iguchi, T.

19. The Anatomical Record, 234, 432-437 (1992)
Mesoderm and neural inductions on newt ectoderm by activin A.
Moriya, N., and Asashima, M.
Develop. Growth Differ., 34(5), 589-594 (1992)
20. Reduction of papilloma size by ultraviolet irradiation in the japanese newt, *Cynops pyrrhogaster*.
Oka, K., Kishi, K., Shiroya, T., Asashima, M., and Pfeiffer, C. J.
J. Comp. Patl., 106, 1-8 (1992)
21. Specific erythroid differentiation of mouse erythroleukemia cells by activins and its enhancement by retinoic acids.
Uchiyama, H., and Asashima, M.
Biochem. Biophys. Res. Commun., 187(1), 347-352 (1992)
22. A new cell line (XTY) from a tumor of *Xenopus laevis*.
Fukui, A., Tashiro, A., Koyama, H., Iwamura, Y., and Asashima, M.
Experientia, 48, 87-91 (1992)
23. Protrusion of notochord from bisected larva during the development of amphibian embryos.
Sato, E., Uchiyama, H., Akahoshi, M., Asashima, M., and Fujita, M.
J. Yokohama City University. (Series Natured Science), 6, 1-8 (1992)
24. A carboxyl-terminal truncated activin receptor mediates activin signals in early *Xenopus* embryos.
Nishimatsu, S., Iwao, M., Nagai, T., Oda, S., Suzuki, S., Asashima, M., Murakami, K., and Ueno, N.
FEBS Letters, 312(2,3), 169-173 (1992)
25. Induction of pronephric tubules by activin and retinoic acid in presumptive ectoderm of *Xenopus laevis*.
Moriya, N., Uchiyama, H., and Asashima, M.
Develop. Growth & Differ., 35(2), 123-128 (1993)
26. Isolation and characterization of *Xenopus* follistatin and activins.
Fukui, A., Nakamura, T., Sugino, K., Takio, K., Uchiyama, H., Asashima, M., and Sugino, H.
Develop. Biol., 159, 131-139 (1993)
27. Expression of GTP-binding protein gene drg in neural tissues of *Xenopus laevis* embryos.
Kumar, S., Iwao, M., Yamagishi, T., Noda, M., and Asashima, M.
Int. J. Develop. Biol., 37, 539-546 (1993)
28. Competence prepatter in the animal hemisphere of the 8-cell-stage *Xenopus* embryo.
Kinoshita, K., Bessho, T., and Asashima, M.
Develop. Biol., 160, 276-284 (1993)
29. Fertilization and embryonic development of *Xenopus* eggs under simulated microgravity in a clinostat.
Koike, H., Takama, A., and Asashima, M.
ISAS Research Note, 539, 1-7 (1993)
30. Growth factors and cell differentiation during early amphibian

- development.
Kinoshita, K., and Asashima, M.
Gann Monograph (ed. By T. Nakamura), 42, 63-78 (1994)
31. Identification of activins A, AB, and B and follistatin proteins in *Xenopus* embryos.
Fukui, A., Nakamura, T., Uchiyama, H., Sugino, H., Sugino, K., and Asashima, M.
Develop. Biol., 163, 279-281 (1994)
 32. Localization of activin and follistatin proteins in the *Xenopus* Oocyte
Uchiyama, H., Nakamura, T., Komazaki, S., Takio, K., Asashima, M., and Sugino, H.
Biochem. Biophys. Res. Commun., 202, 484-489 (1994)
 33. Control of cell differentiation and morphogenesis in amphibian development
Fukui, A., and Asashima, M.
Int. J. Develop. Biol., 38, 257-266 (1994)
 34. A view of amphibian embryology research in Japan through the scientific biography of Professor Osamu Nakamura
Asashima, M.
Int. J. Dev. Biol., 38, 155-165 (1994)
 35. Ultrastructure of the olfactory organ of the newt, *Cynops pyrrhogaster*
Jones, F. M., Pfeiffer, C. J., and Asashima, M.
Annals of Anatomy, 176, 269-275 (1994)
 36. HMG-X, a *Xenopus* gene encoding an HMG1 homolog, is abundantly expressed in the developing nervous system
Kinoshita, M., Hatada, S., Asashima, M., and Noda, M.
FEBS Letter, 352, 191-196 (1994)
 37. Mesoderm induction during early amphibian development.
Asashima, M.
Develop. Growth & Differ., 36(4), 343-355 (1994)
 38. *In vitro* control of the embryonic form of *Xenopus laevis* by activin A: Time and dose-dependent inducing properties of activin-treated ectoderm.
Ariizumi, T., and Asashima, M.
Develop. Growth & Differ., 36(5), 499-507 (1994)
 39. Formation of otoconia in the Japanese red-bellied newt, *Cynops Pyrrhogaster*
Wiederhold, M. L., Yamashita, M., Larsen, K., Asashima, M.
Adv. Space Res., 14(8), 327-330 (1994)
 40. Identification of a heparin-binding, mesoderm-inducing peptide in the swim-bladder of the red seabream, *Pagrus major*: a probable fish fibroblast growth factor.
Suzuki, T., Kurokawa, T., and Asashima, M.

41. Fish Physiology and Biochemistry, 13(4), 343-352 (1994)
Non-invasive assessment of otolith formation during development of the Japanese red-bellied newt, *Cynops pyrrhogaster*
Koike, H., Nakamura, K., Nishimura, K., Kashima, I., Wiederhold, M. L., and Asashima, M.
Hearing Research, 88, 206-214 (1995)
42. Head and trunk-tail organizing effects of the gastrula ectoderm of *Cynops pyrrhogaster* after treatment with activin A
Ariizumi, T., and Asashima, M.
Roux' Archives Develop. Biol., 204, 427-435 (1995)
43. Onset of the competence to respond to activin A in isolated eight-cell stage *Xenopus* animal blastomeres.
Kinoshita, K., Bessho, T., and Asashima, M.
Develop. Growth Differ., 37, 303-309 (1995)
44. Activin Induces the Expression of the *Xenopus* Homologue of Sonic Hedgehog during Mesoderm Formation in *Xenopus* Explants.
Yokota, C., Mukasa, T., Higashi, M., Odaka, A., Muroya, K., Uchiyama, H., Eto, Y., Asashima, M. and Momoi, T.
Biochem. Biophys. Res. Commun., 207(1), 1-7 (1995)
45. Development of the otolith and semicircular canals in the Japanese red-bellied newt, *Cynops pyrrhogaster*.
Wiederhold, M. L., Yamashita, M., Larsen, K. A., Batten, J. S., Koike, H., and Asashima, M.
Hearing Research, 84, 41-51 (1995)
46. Restricted expression of *Xenopus* midkine gene during early development
Sekiguchi, K., Yokota, C., Kaname, T., Q.-Wen - Fan, Kadomatsu, K., Asashima, M., and Muramatsu, T.
J. Biochemistry, 118, 94-100 (1995)
49. Cytopathologic observation of the lung of adult newts (*Cynops pyrrhogaster*) on-board the space shuttle, Columbia, during the second international microgravity laboratory experiments.
Pfeiffer, C. J., Yamashita, M., Izumi-Kurokawa, A., Koike, H., and Asashima, M.
J. Submic. Cytol. Path., 27, 501-509 (1995)
50. Astronewt: Early development of newt in space
Mogami, Y., Imamizo, M., Yamasita, M., Izumi-Kurotani, A., Wiederhold, M. L., Koike, H., and Asashima, M.
Adv. Space Res., 17, 257-263 (1995)
51. Identification of a *Xenopus* glutamine synthetase gene abundantly expressed in the embryonic nervous system but not in adult brain.
Hatada, S., Kinoshita, M., Noda, M., and Asashima, M.
FEBS Letters, 371, 287-292 (1995)
52. Control of the embryonic body plan by activin during amphibian development

- Ariizumi, T., and Asashima, M.
Zool. Science, 12, 509-521 (1995)
53. Effect of activin and lithium on isolated *Xenopus* animal blastomeres and response alteration at the midblastula transition.
Kinoshita, K., and Asashima, M.
Development, 121, 1581-1589 (1995)
 54. Comparison of Mesoderm-Inducing Activity with Monomeric and Dimeric Inhibin Alpha and Beta-A Subunits on *Xenopus* Ectoderm
Nakano, H., Uchiyama, H., Fukui, A., Sugino, H., and Asashima, M.
Hormone Research, 44, 15-22 (1995)
 55. Occurrence of Immunoreactive Activin/Inhibin β B in Gonadotrophs, Thyrotrophs, and Somatotrophs of the *Xenopus* Pituitary
Uchiyama, H., Komazaki, S., Asashima, M. and Kikuyama, S.
General and Comparative Endocrinology, 102, 1-10 (1996)
 56. Determination, induction and pattern formation in early amphibian embryos.
Tiedemann, H., Asashima, M., Born, J., Grunz, H., Knoehel, W., and Tiedemann, H.
Develop. Growth Differ., 38, 233-246 (1996)
 57. Sequential Gene Expression during pronephric Tubule Formation *In vitro* in *Xenopus* Ectoderm
Uochi, T., and Asashima, M.
Develop. Growth Differ., 38 (6), 625-634 (1996)
 58. cDNA Cloning and Expression of the *Xenopus* leavis Vitellogenin receptor.
Okabayashi, K., Shoji, H., Nakamura, T., Hashimoto, O., Asashima, M., and Sugino, H.
Biochem. Biophys. Res. Commun., 224, 406-413 (1996)
 59. Activin treated urodele ectoderm: a model experimental system for cardiogenesis.
Ariizumi, T., Komazaki, S., Asashima, M., and G.M. Malacinski.
Int. J. Devel. Biol., 40, 715-718 (1996)
 60. Conversion of ectoderm into a neural fate by ATH-3, a ventabrate basic helix-loop-helix gene homologous to *Drosophila* proneural gene atonal.
Takabayasi, K., Takahasi, S., Yokota, C., Tsuda, H., Nakanishi, S., Asashima, M., and Kageyama, R.
EMBO Journal 16(2), 384-395 (1997)
 61. An Essay on the Similarities and Differences Between Inductive Interactions in Anuran and Urodele Embryos.
Malacinski, G. M., Bessho, T., Yokota, C., Fukui, A., and Asashima, M.
Cellular and Molecular Life Science, 53, 410-417 (1997)
 62. A novel gene encoding a ferredoxin reductase-related protein expressed in the neuroectoderm in *Xenopus* neurula.

- Hatada, S., Kinoshita, M., Sakumoto, H., Nishihara, R., Noda, M. and Asashima, M.
Gene, 194/2, 297-299 (1997)
63. *Xenopus* FK506-binding protein homolog induces a secondary axis in frog embryos, which is inhibited by coexisting BMP-4 signaling.
Nishinakamura, R., Matsumoto, Y., Uochi, T., Asashima, M. and Yokota, T.
Biochem. Biophys. Res. Commun., 239, 585-591 (1997)
64. The Na⁺, K⁺-ATPase a subunit requires gastrulation in the *Xenopus* embryo.
Uochi, T., Takahashi, S., Ninomiya, H., Fukui, A. and Asashima, M.
Develop. Growth Differ., 39, 571-580 (1997)
65. An interferon regulatory factor-related gene (xIRF-6) is expressed in the posterior mesoderm during the early development of the *Xenopus laevis*.
Hatada, S., Kinoshita, M., Takahashi, S., Nishihara, R., Sakumoto, H., Fukui, A., Noda, M. and Asashima, M.
Gene, 203, 183-188 (1997)
66. Bio experiment on space flyer unit: effects of gravity on early development of Japanese red bellied newt
Yamashita, M., Isumi-Kurotani, A., Imamizo, M., Mogami, Y., Koike, H., Komazaki, S. and asashima, M.
J. Space Technology and Science, 13(2), 12-17 (1997)
67. Evidence that far infrared radiation promotes growth of *Xenopus laevis*
Shiuruba, R., Hirabayashi, T., Kiyokawa, S., Fukui, A., Miyanaga, Y., Kojima, I., and Asashima, M.
Biological Sciences in Space, Vol.11, No.4, 311-312 (1997)
68. Midkine Counteracts the Activin Signal in Mesoderm Induction and Promotes Neural Formation.
Yokota, C., Takahashi, S., Eisaki, A., Asashima, M., S., Akhter, S., Muramatsu, T. and Kadomatsu, K.
J. Biochemistry., 123, 339-346 (1998)
69. Cloning and expression pattern of *Xenopus* prx-1(Xprx-1) during embryonic development.
Takahashi, S., Uochi, T., Kawakami, Y., Nohno, T., Yokota, C., Kinoshita, K. and Asashima, M.
Develop. Growth Deffer. 40, 97-104 (1998)
70. Induction of blood cells in *Xenopus* embryo explants.
Miyanaga, Y., Shiurba, R., Nagata, S., Pfeiffer, J., and Asashima, M.
Develop. Genes and Evolution., 207, 417-426 (1998)
71. Pattern of gene expression in the core of Spemann's organizer and activin-treated ectoderm in *Cynops pyrrhagaster*.
Yokota, C., Ariizumi, T. and Asashima, M.
Develop. Growth Deffer. 40, 335-341 (1998)

72. Axil, a member of axin family, interacts with both GSK-3 β and β -catenin and inhibits axis formation of *Xenopus* embryo.
Yamamoto, H., Kishida, S. Uochi, T., Ikeda, S., Koyma, S., Asashima, M. and Kikuchi, A.
Molecular and Cellular Biology, 18, 2867-2875 (1998)
73. XCIRP (*Xenopus* homolog of cold-inducible RNA binding protein) is expressed transiently in developing pronephros and neural tissue
Uochi, T. and Asashima, M.
Gene, 211/2, 245-250 (1998)
74. Regulation of the Xmyf-5 and XmyoD Expression Pattern during Early *Xenopus* Development.
Takahashi, S., Esumi, E., Nabeshima, Y., and Asashima, M.
Zool. Science, 15, 231-238 (1998)
75. Activin-treated ectoderm has complete organizing center activity in *Cynops* embryos.
Ninomiya, H., Ariizumi, T., and Asashima, M.
Develop. Growth Differ., 40, 199-208 (1998)
76. Neural induction in embryos
Tiedemann, H., Asashima M., Grunz, H., Knochel, W., and Tiedemann, H.
Develop. Growth Differ., 40, 363-376 (1998)
77. *In vitro* Control of Embryonic Axis Formation by Activin A, Concanavalin A and Retinoic Acid in *Xenopus laevis*
Moriya, N., Yokota, C., Ariizumi, T., and Asashima, M.
Zool. Science, 15, 879-886 (1998)
78. Activin-Treated Urodele Animal Caps: I. Mesoderm and Endoderm Differentiation of Salamander Animal Caps
Ariizumi, T., Takano, K., Ninomiya, H., and Asashima, M.
Zool. Science, 15, 887-892 (1998)
79. Molecular cloning of XNLRR-1, a *Xenopus* homolog of mouse neuronal leucine-rich repeat protein expressed in the developing *Xenopus* nervous system
Hayata, T., Uochi, T., and Asashima, M.
Gene, 221, 159-166 (1998)
80. cDNA Cloning and Distribution of the *Xenopus* Follistatin-Related Protein
Okabayashi, K., Shoji, H., Onuma, Y., Nakamura, T., Nose, K., Sugino, H., and Asashima, M.
Biochem. Biophys. Res. Commun., 254, 42-48 (1999)
81. A Model system for Organ Engineering: Transplantation of *In vitro* Induced Pronephros.
Chan, T., Ariizumi, T., and Asashima, M.
Naturwissenschaften, 86, 224-227 (1999)
82. Blood cell induction in *Xenopus* animal cap explants: effects of fibroblast growth factor, bone morphogenetic proteins, and activin

- Miyanaga, Y., Shiurba, R., and Asashima, M.
Dev. Genes Evol., 209, 69-76 (1999)
83. Role of Activin and Other Peptide Growth Factors in Body Patterning in the Early Amphibian Embryo
Asashima, M., Kinoshita, K., Ariizumi, T., and Malacinski, G. M.
Int. Rev. Cytology, 191, 1-52 (1999)
 84. Cytochalasin B inhibits morphogenetic movement and muscle differentiation of activin-treated ectoderm in *Xenopus*
Tamai, K., Yokota, C., Ariizumi, T., and Asashima, M.
Develop. Growth Differ., 41, 41-49 (1999)
 85. Peptide growth factors in amphibian embryogenesis: intersection of modern molecular approaches with traditional inductive interaction paradigms
Asashima, M., Yokota, C., Takahashi, S., Lau, C. L. and Malacinski, G. M.
Int. J. Dev. Biol., 43, 1-10 (1999)
 86. Changes in the adhesive properties of dissociated and reaggregated *Xenopus laevis* embryo cells
Kuroda, H., Sakumoto, H., Kinoshita, K. and Asashima, M.
Develop. Growth Differ., 41, 283-291 (1999)
 87. Activin-treated Urodele Animal Caps: II. Inductive Interactions in Newt Animal Caps After Treatment with Activin A
Ariizumi, T., Komazaki, S. and Asashima, M.
Zoological Science, 16(1), 115-124 (1999)
 88. Endoderm differentiation and inductive effect of activin-treated ectoderm in *Xenopus*
Ninomiya, H., Takahashi, S., Tanegashima, K., Yokota, C. and Asashima, M.
Develop. Growth Differ. 41, 391-400 (1999)
 89. Activin Incorporation into Vitellogenic Oocytes of *Xenopus Laevis*
Fukui, A., Shiurba, R., Asashima, M.
Cellular and Molecular Biology, 45(5), 545-554 (1999)
 90. Expression of Brachyury-like T-box transcription factor, Xbra3 in *Xenopus* embryo
Hayata, T., Eisaki, A., Kuroda, H., Asashima, M.
Dev. Genes Evol., 209, 560-563 (1999)
 91. Expression of *Xenopus* T-box transcription factor, Tbx2 in *Xenopus* embryo
Hayata, T., Kuroda, H., Eisaki, A. and Asashima, M.
Dev. Genes Evol., 209, 625-628 (1999)
 92. Bioassays of Inductive Interactions in Amphibian Development
Ariizumi, T., Takano, K., Asashima, M and Malacinski, G.M.
Methods in Molecular Biology 135: Developmental Biology Protocols 1, 89-112 (1999)
 93. Molecular Cloning of a Novel *Xenopus* spalt Gene (Xsal 3)

- Onuma, Y., Nishinakamura, R., Takahashi, S., Yokota, T. and Asashima, M.
Biochemical and Biophysical Research Communications, 264, 151-156 (1999)
94. Evidence that Far-Infrared Radiation Promotes Growth of *Xenopus Laevis*
Shiurba, R., Hirabayashi, T., Kiyokawa, S., Fukui, A., Miyanaga, Y., Kojima, I. and Asashima, M.
Adv. Space Res., 23(12), 2041-2044 (1999)
 95. *In vitro* control of organogenesis and fundamental embryonic form by the peptide growth factor activin
Ariizumi, T., Asashima, M.
J. Artif Organs 2, 117-123 (1999)
 96. Activation of Stat3 by cytokine receptor gp130 ventralizes *Xenopus* embryos independently of BMP-4
Nishinakamura, R., Matsumoto, Y., Matsuda, T., Heike, T., Asashima, M. and Yokota, T.
Dev. Biol., 216, 481-490 (1999)
 97. Bioassays for Studying the Role of the Peptide Growth Factor Activin in Early Amphibian Embryogenesis
Asashima, M., Ariizumi, T., Takahashi, S. and Malacinski, G.M.
Methods in Molecular Biology 136: Developmental Biology Protocols II 15-26 (1999)
 98. Effect of TGF- β on morphogenesis in rat salivary gland-derived RSMG-1 Cells
Furue, M., Okamoto, T. and Asashima, M.
Tiss. Cult. Res. Commun., 18:339-343 (1999)
 99. Cloning a novel developmental regulating gene, *Xotx5* : its potential role in anterior formation in *Xenopus laevis*
Kuroda, H., Hayata, T., Eisake, A. and Asashima M.
Develop. Growth Differ., 42, 87-93 (2000)
 100. *In vitro* organogenesis of pancreas in *Xenopus laevis* dorsal lips treated with retinoic acid
Moriya, N., Komazaki, S. and Asashima, M.
Develop. Growth Differ., 42, 175-185 (2000)
 101. FGF Plays a Critical Role in SM22 α Expression during *Xenopus* Embryogenesis
Oka, T., Shiojima, I., Monzen, K., Kudoh, S., Hiroi, Y., Shiokawa, K., Asashima, M., Nagai, R., Yazaki, Y. and Komuro, I.
Arteriosclerosis, Thrombosis, and Vascular biology, 907-914 (2000)
 102. Gene expression pattern Cloning and expression pattern of a *Xenopus* pronephros-specific gene, XSMP-30
Sato, A., Asashima, M., Yokota, T. and Nishinakamura, R.
Mech. Dev., 92, 273-275 (2000)
 103. *In vitro* control of organogenesis and body patterning by activin

- during early amphibian development
Asashima, M., Ariizumi, T. and Malacinski, G.M.
Comparative Biochemistry and Physiology, 126, 169-178 (2000)
104. The renaissance in amphibian embryology
Malacinski, G.M., Ariizumi, T. and Asashima, M.
Comparative Biochemistry and Physiology, 126, 179-187 (2000)
105. Development of the embryonic kidney
Chan, Te-Chuan and Asashima, M.
Clin. Exp. Nephrol, 4, 1-10 (2000)
106. Expression of the *Xenopus* GTP-binding protein gene Ran during embryogenesis
Onuma, Y., Nishihara, R., Takahashi, S., Tanegashima, K., Fukui, A. and Asashima, M.
Development Genes and Evolution, 210, 325-327 (2000)
107. XSIP1, a Member of Two-Handed Zinc Finger Proteins, Induced Anterior Neural Markers in *Xenopus laevis* Animal Cap
Eisaki, A., Kuroda, H., Fukui, A. and Asashima, M.
Biochemical and Biophysical Res. Com., 271, 151-157 (2000)
108. The evolutionarily conserved porcupine gene family is involved in the processing of the Wnt family
Tanaka, K., Okabayashi, K., Asashima, M. Perrimon, N. and Kadowaki, T.
Eur. J. Biochem., 267, 1-13 (2000)
109. Effect of rat Axin domains on axis formation in *Xenopus* embryos
Fukui, A., Kishida, S. Kikuchi, A. and Asashima, M.
Develop. Growth & Differ., 42(5), 489-498 (2000)
110. *In vitro* pancreas formation from *Xenopus* ectoderm treated with activin and retinoic acid
Moriya, N., Komazaki, S., Takahashi, S., Yokota, C. and Asashima, M.
Develop. Growth & Differ., 42(6), 593-602 (2000)
111. Inhibition of Wnt Signaling Pathway by A Novel Axin-binding Protein
Kadoya, T., Kishida, S., Fukui, A., Hinoi, T., Michiue, T., Asashima, M. and Kikuchi, A.
J. Biol. Chem., 275(47), 37030-37037
112. Expression cloning of Xantivin, a *Xenopus* lefty/antivin related gene, involved in the regulation of activin signaling during mesoderm induction
Tanegashima, K., Yokota, C., Takahashi, S. and Asashima, M.
Mech. Develop., 99, 3-14 (2000)
113. Isoleucine Prevents rat salivary gland epithelial cells from Apoptosis in Serum-Free Culture
M. Furue, T. Okamoto, S. Koshika and M. Asashima
In vitro Cell. Dev. Biol. -Animal 36:287-289, May 2000, 2000 Society

- for *In vitro* Biology
114. A Novel β -Catenin Binding Protein Inhibits β -Catenin-dependent Tcf Activation and Axis Formation
Sakamoto, I., Kishida, S., Fukui, A., Kishida, M., Yamamoto, H., Hino, S., Michiue, T., Takada, S., Asashima, M. and Kikuchi, A.
J. Biol. Chem., 275(42), 32871-32878 (2000)
 115. Meiotic maturation induces animal-vegetal asymmetric distribution of aPKC and ASIP/PAR-3 in *Xenopus* oocytes
M. Nakaya, A. Fukui, Y. Izumi, K. Akimoto, M. Asashima and S. Ohno
Development, 127, 5021-5031 (2000)
 116. Two novel nodal-related genes initiate early inductive events in *Xenopus* Nieuwkoop center
S. Takahashi, C. Yokota, K. Takano, K. Tanegashima, Y. Onuma, J. Goto and M. Asashima
Development, 127, 5319-5329 (2000)
 117. Spontaneous Thyroid-containing Teratoma Associated with Impaired Development in the African Clawed Frog, *Xenopus laevis*
S.W. Cheong, A. Fukui, M. Asashima and C.J. Pfeiffer
J. Comp. Path., 123, 110-118 (2000)
 118. A Role for Xlim-1 in Pronephros Development in *Xenopus laevis*
Te-chuan Chan, S. Takahashi and M. Asashima
Develop. Biol., 228, 256-269 (2000)
 119. Spemann's influence on Japanese Developmental Biology.
Asashima, M., Okada, S. Tokindo.
J. Dev. Biol, 45, 57-65 (2001)
 120. *In vitro* induction systems for analyses of amphibian organogenesis and body patternig.
Ariizumi, T., Asashima, M.
J. Dev. Biol, 45, 273-279 (2001)
 121. Dome Formation and Tubule Morphogenesis by *Xenopus* Kidney A6 Cell Cultures Exposed to Microgravity Simulated with A 3D-Clinostat and to Hypergravity.
Junji Ichigi and Makoto Asashima
In vitro Cellular and Developmental Biology·Animal., 37, 31-44 (2001)
 122. Activin A Induces Expression of Rat Sel-1/mRNA, a Negative Regulator of Notch Signaling, in Rat Salivary Gland-Derived Epithelial Cell.
Furue M, Zhang Y, Okamoto T, Hata RI, Asashima M.
Biochemical and Biophysical Research Communications., 282, 745-749

- (2001)
123. Inhibition of the Signaling Pathway by the PR61 Subunit of Protein Phosphatase 2A.
Hideki Yamamoto, Toshihide Hinoi, Tatsuo Michiue, Akimasa Fukui, Hirofumi Usui, Veerle, Janssens, Christine Van Hoof, Jozef Goris, Makoto Asashima, and Akira Kikuchi
The Journal of Biological Chemistry, 276, 29, 26875-26882 (2001)
124. Murine homolog of SALL1 is essential for ureteric bud invasion in kidney development.
Ryuichi Nishinakamura, Yuko Matsumoto, Kazuki Nakao, Kenji Nakamura, Akira Sato, Neal G. Copeland, Debra J. Gilbert, Nancy A. Jenkins, Sheila Scully, David L. Lacey, Motoya Katsuki, Makoto Asashima and Takashi Yokota
Development, 128, 3105-3115 (2001)
125. Synergistic Activation of the Wnt Signaling Pathway by Dvl and Casein Kinase I ϵ . Michiko Kishida, Shin-ichiro Hino, Tatsuo Michiue, Hideki Yamamoto, Shosei Kishida, Akimasa Fukui, Makoto Asashima, and Akira Kikuchi
The Journal of Biological Chemistry, 276 (35) 33147-33155 (2001)
126. Pluripotent cells (stem cells) and their determination and differentiation in early vertebrate embryogenesis.
H. Tiedemann, M. Asashima, H. Grunz and W. Knochel
Develop. Growth Differ., 43, 469-502 (2001)
127. Inhibition of the Wnt Signaling Pathway by Idax, a Novel Dvl-Binding Protein.
S-I. Hino, S. Kishida, T. Michiue, A. Fukui, I. Sakamoto, S. Takada, M. Asashima A. Kikuchi
Mol. and Cell. Biol., 21, 330-342 (2001)
128. Cloning and characterization of the *Xenopus laevis* p8 gene.
T. Igarashi, H. Kuroda, S. Takahashi and M. Asashima
Develop. Growth Differ., 43, 693-698 (2001)
129. Overexpression of the secreted factor Mig30 expressed in the Spemann organizer impairs morphogenetic movements during *Xenopus* gastrulation.
Tadayoshi Hayata, Kosuke Tanegashima, Shuji Takahashi, Asako Sogame, and Makoto Asashima

- Mech. Develop., 112, 37-51 (2002)
130. Multipul nodal-Related Genes Act Coordinately in *Xenopus* Embryogenesis
Yasuko Onuma, Shuji Takahashi, Chika Yokota, and Makoto Asashima
Devel. Biol., 241. 94-105 (2002)
 131. Conservation of Pax 6 function and upstream activation by Notch signaling in eye development of frogs and flies.
Onuma Y, Takahashi S, Asashima M, Kurata S, Gehring WJ.
Proc Natl Acad Sci., 99. 2020-2025 (2002)
 132. Role of BMP-4 in the Inducing Ability of the Head Organizer in *Xenopus laevis*.
Ayako Sedohara, Akimasa Fukui, Tatsuo Michiue and Makoto Asashima
Zoological Science, 19 (1), 67-80 (2002)
 133. Synthesis and release of activin and noggin by cultured human amniotic epithelial cells.
Satoru Koyano, Akimasa Fukui, Saiko Uchida, Kazuto Yamada, Makoto Asashima and Norio Sakuragawa
Develop. Growth Differ., 44, 103-112 (2002)
 134. *In vitro* induction of the pronephric duct in *Xenopus* explants.
Kenji Osafune, Ryuichi Nishinakamura, Shinji Komazaki and Makoto Asashima
Develop. Growth Differ., 44, 161-167 (2002)
 135. Axial Protocadherin is a Mediator of Prenotochord Cell Sorting in *Xenopus*.
Hiroki Kuroda, Masafumi Inui, Kaoru Sugimoto, Tadayoshi Hayata and Makoto Asashima
Developmental Biology, 244, 267-277 (2002)
 136. Experimental split cord malformations.
T. Emura, M. Asashima, M. Furue and K. Hashizume
Pediatr. Neutrosurg., 36, 229-235 (2002)
 137. Desumoylation activity of Axam, a novel Axin-binding protein, is involved in downregulation of β -catenin.
T. Kadoya, H. Yamamoto, T. Suzuki, A. Yukita, A. Fukui, T. Michiue, T. Asahara, K. Tanaka, M. Asashima and A. Kikuchi

- Molecular and Cellular Biology, 22(11), 3803-3819 (2002)
138. Nuclear localization of Duplin, a β -catenin-binding protein, is essential for its inhibitory activity on the Wnt signaling pathway.
M. Kobayashi, S. Kishida, A. Fukui, T. Michiue, Y. Miyamoto, T. Okamoto, Y. Yoneda, M. Asashima and A. Kikuchi
J. Biol. Chem., 277(8), 5816-5822 (2002)
139. Role of the thrombopoietin (TPO)/Mpl system: c-Mpl-like molecule/TPO signaling enhances early hematopoiesis in *Xenopus laevis*.
M. Kakeda, J. Kyuno, T. Kato, M. Nishikawa and M. Asashima
Develop. Growth Differ., 44, 63-75 (2002)
140. Molecular cloning and characterization of dullard: a novel gene required for neural development.
R. Satow, T. Chan and M. Asashima
B. B. R. C., 295, 85-91 (2002)
141. The second largest subunit of mouse DNA polymerase ϵ , DPE2, interacts with SASP18 and recruits the Sin3 co-repressor protein to DNA
Wada, M., Miyazawa, H., Wang, R., Mizuno, T., Sato, A., Asashima, M. and Hanaoka, F.
J. Biochem., 131, 307-311 (2002)